Buccal swab stability comparison using either BuccalFix, SGC Dri-Capsules or no stabilisation in a 5ml tube over 4 or 7 days at room temperature.

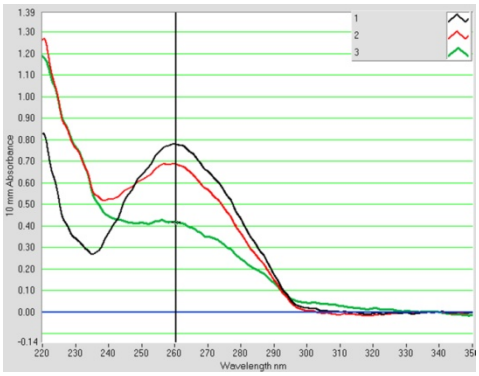
Sets of 3 swabs were taken at the same time, swab 1 was stored in tube containing 0.5ml BuccalFix swab stabilisation buffer, swab 2 was stored in a 5ml tube with an Isohelix SGC Dri-Capsule and swab 3 was placed in a sealed 5ml tube with no stabilisation. All 3 swabs were stored at room temperature for 4 days before isolation using the BuccalPrep DNA Isolation kit (or for swab 1 where the swab was already in the lysis buffer, the BuccalFix DNA isolation kit).

All samples were resuspended in 150ul TE buffer and analysed by Nanodrop, Qubit dsDNA BR assay and whole DNA agarose gel to compare yields, purity and to examine the integrity of the isolated DNA.

Results:

Nanodrop data:

Sample	ng/ul	A260	A280	260/280	260/230	340 raw
1 – BFX	38.93	0.779	0.434	1.79	2.08	0.075
2 – SGC	34.35	0.687	0.379	1.81	1.01	0.106
3 – u/s	20.90	0.418	0.247	1.69	0.47	0.370



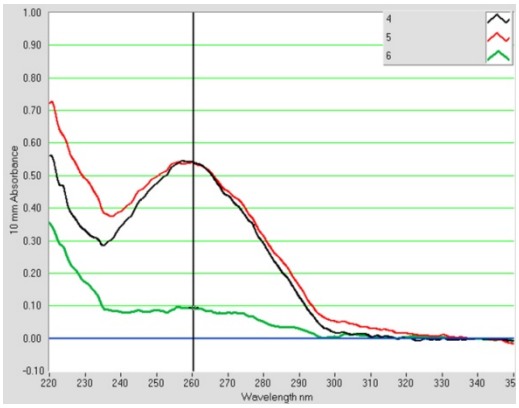
Yield and purity are best with the BuccalFix stabilised swab. The swab stabilised with the Dri-Capsule has the same A260/280 ratio as the BuccalFix swab but a lower a260/230 ratio. Yield is very slightly lower. The un-stabilised swab shows both reduced yield and purity and no defined peak at 260nm.

Quant-iT dsDNA BR							
Sample	Concentration in the Qubit		uL used	Dilution	Sample Concentration		Total ug
1 – BFX	1.42	ug/mL	10	20	28.4	ug/mL	4.26
2 – SGC	1.16	ug/mL	10	20	23.1	ug/mL	3.47
3 – un stabilised	0.389	ug/mL	10	20	7.78	ug/mL	1.17

Whole DNA on an agarose gel (data not shown) from swab 1 showed a high molecular weight (>10Kb) band, with very little smear of lower molecular weight material. The DNA from swab 2 showed a bright band of high molecular weight material with some smear of lower molecular weight material down the gel as expected, indicating a small amount of DNA has degraded during the drying process. There was only a faint band or high molecular weight DNA present in the sample from swab 3, with a smear of DNA from approx. 500bp downwards indicating a significant proportion of DNA in this sample is almost entirely degraded.

From a separate experiment, a set of swabs were taken (swabs 4, 5 and 6) and stored as described above for 7 days at room temperature before isolation through the BuccalPrep or BuccalFix kit. The samples were resuspended in 200ul TE buffer and analysed by Nanodrop, Qubit dsDNA BR assay, whole DNA on agarose gel and DQC quality check PCR kit to check DNA yields, purity and integrity.

Results:



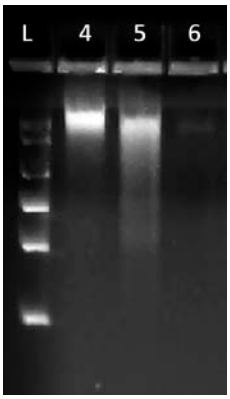
Nanodrop data

Sample	ng/ul	A260	A280	260/280	260/230	340 raw
4 - BFX	27.08	0.542	0.292	1.85	1.63	0.018
5 - SGC	26.94	0.539	0.314	1.71	1.10	0.187
6 – u/s	4.68	0.094	0.051	1.85	0.54	0.071

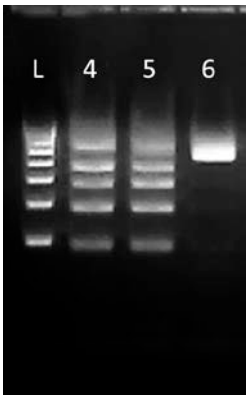
Nanodrop results are similar to the 4 day samples except the un-stabilised swab, sample 6, has a much lower yield and purity as measured by A260/230. The scan itself indicates a very poor quality sample.
Qubit results below confirm a very low yield in the un-stabilised swab sample.

Quant-iT dsDNA BR							
Sample	Concentration in the Qubit		uL used	Dilution	Sample Concentration		Total ug
4 – BFX	1.02	ug/mL	10	20	20.3	ug/mL	4.06
5 – SGC	0.782	ug/mL	10	20	15.6	ug/mL	3.12
6 – un stabilised	0.059	ug/mL	10	20	1.18	ug/mL	0.236

Whole DNA on 2.2% agarose gel



DQC Quality Check PCR



Only the internal control band of 500bp amplifies in sample 6 indicating no amplifiable DNA in the sample.
Swabs 4 and 5 give the expected results for intact DNA, amplifying 5 bands of 100, 200, 300, 400 and 600bp as well as the internal 500bp control band.

Conclusions:

Significant DNA degradation can occur on buccal swabs stored for short periods of time without stabilisation, to the extent that obvious degradation has already occurred by day 4, and that no amplifiable DNA may be present after a period of 7 days. Both BuccalFix stabilisation buffer and use of Isohelix Dri-Capsules prevents or reduces any degradation to a minimum, enabling intact high molecular weight to be isolated form buccal swabs stored at room temperature for either short or long periods of time.

Note: Results may vary from individual to individual depending on how well the swabbing instructions have been followed as well as the individual's typical microbial population naturally present in the buccal cavity.